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The polymerase chain reaction technique as a specific and sensitive detection method for *Aeromonas salmonicida* and *Aeromonas sobria* in natural ecosystems (water, sediment, and fish)

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Aquaculture provides about 12% of the food man obtains from the waters of the earth. The world's tropics with their climate present greater developmental potential for aquaculture than the temperate zone, and thus promise to be especially valuable in policies on improving the diets of developing nations (Bardach, 1985). The economic and nutritional importance of aquaculture to developing countries is recognized by the fact that it does not only provide cheap, good quality fish-food, but also earns substantial foreign exchange (exports of high-value products), creates employment, particularly in economically depressed coastal and remote regions, and contributes to the household economy of farmers. In 1988, total world aquaculture production was valued at US $22.454 billion, 82% of which was produced in the developing countries of Asia, South America, and Africa (FAO, 1990).

There are many constraints to the development of aquaculture in developing countries, one of the major problems being fish and shrimp diseases. The International Foundation of Science noted that of its more than 100 aquaculture grants on this date (1995) only 13 are concerned with fish health problems. Two years ago this was even less, when only 2 of the 100 grants were involved in fish health.

However, studies about the causes of the diseases and their ecological effects in natural fish populations/ecosystems are extremely difficult to quantify. The identifications of pathogens have largely been confined to classical bacterial culture techniques. The methods involved in the identification of pathogens are very time-consuming (Austin and Priest, 1986), and provide sometimes inconclusive results (Kinne, 1977).

Based on the amount of investment in aquaculture enterprises at the moment, there is a need for a rapid, sensitive, and specific technique for identifying bacteria that are responsible for disease outbreaks in fish and shrimp cultures. With this note, we are drawing attention to a new molecular biological method which can be used for identifying all kinds of species, also pathogenic bacteria.

One of the latest and an already very popular technique that makes use of the hybridization properties of the DNA, is the polymerase chain reaction or PCR technique. The PCR is able to amplify very small amounts of specific DNA, even in mixtures of biological samples such as blood. The technique is so sensitive that a single DNA molecule can be amplified and the copied genes can be routinely extracted as distinct bands on agarose gel (Erlich, 1989). Speed and ease are not the only advantage PCR provides; it can work with virtually any genetic starting material, no matter how scanty or degraded (Appenzeller, 1990). The processes involved are: (1) extraction of the genetic material, (2) use of the known sequences of rDNA to amplify the bacterial DNA in a specific manner, and (3) the amplified sequence use of electrophoresis to reveal as a distinct band (Erlich, 1989; Innis *et al.*, 1990; Barry *et al.*, 1990).

Using this process, a diagnostic method was developed for the detection of *Aeromonas salmonicida* and *Aeromonas sobria* in ulcers of flounders, in water, and in sediment samples from an area of the Dutch Wadden Sea in May 1991. Different methods of DNA extraction and PCR protocols were tested to optimize the identification process and to minimize the costs. The sensitivity of the PCR test was compared with classical culture methods. Finally, a confirmation test for the optimized method was carried out on field samples by comparing it with the results of pure cultures.

The results revealed that the method which made use of ultrasonics was the most promising. The sensitivity of the optimized PCR protocol for both species was 10 pg of DNA, while in Petri dish cultures there is visually an absence of these species. Results of the confirmation test for species-specificity showed that when internal primers for *A. salmonicida* are applied to the DNA of *A. salmonicida* from pure cultures, positive bands specific for this species are present, but absent when applied to the

DNA of *A. sobria*. Similar results were observed with internal primers for *A. sobria*. The consistency of the results in tests between the pure cultures and field samples is encouraging. *A. salmonicida* and *A. sobria* could be detected in ulcers of flounders, in the water, and in the sediment. The PCR technique is so sensitive that, in principle, the presence of one specific DNA string in the extract is enough to be amplified by the PCR.

Much research still has to be done, for example research about the estimation of concentrations of pathogens and about concentrations which will cause an outbreak of a disease. We have to keep in mind that the outbreak of a disease does not just depend on the presence of pathogens but also on the physiological condition of the host. For practical reasons it would be interesting to use the high sensitivity of the PCR reaction to estimate the presence of pathogens, even before the pathogens reach levels which start to affect the host. In that way preventative therapies could be applied at the right moment.

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